Methods for Collecting Eye Gnats (Diptera: Chloropidae)

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ABSTRACT

Reviews methods used and described between 1928 and 1977 for collecting *Hippelates* and *Siphunculina* (eye gnats). Considers collection of eggs, larvae, pupae, and adults. Reviews netting, aspirators, baits, and traps. Brief reference is made to population dynamics (dispersal and responses to the environment) and to rearing and handling various species. Reviews relationship of *Hippelates* and *Siphunculina* to disease. Includes 110 literature citations.

KEYWORDS: *Hippelates*, *Siphunculina*, Chloropidae, Diptera, eye gnats, collection, rearing, traps, disease, pinkeye, yaws, anaplasmosis, nephritis, pinta

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METHODS FOR COLLECTING EYE GNATS (FAMILY CHLOROPIDAE)

By William M. Rogoff[1]

INTRODUCTION

Eye gnats are best known as pests of humans, though sharp delineation between "pests of humans" and "pests of livestock" is not always possible. The emphasis of published work on eye gnats is almost entirely oriented toward the public health and pests of humans aspects.

Only three genera (*Hippelates*, *Siphunculina*, and *Oscinella*) and a small number of species of Chloropidae have had serious attention other than as pests of crops, though the family is large and cosmopolitan. In India, Sri Lanka (Ceylon), and Java, the "eye fly," *Siphunculina funicola* de Meijere, has been especially disturbing and has been credited as the primary vector of epidemic conjunctivitis commonly referred to as pink eye. *Oscinella aharonii* Duda from Egypt and Sudan to India and *O. sziladyi* Duda from Bulgaria seem to fill a similar niche in those regions. In the United States, *Hippelates collusor* (Townsend) in the Southwest and *H. pusio* Loew in the Southeast fill a comparable role.

Payne et al. (*79*)[2] showed that *H. pusio* are capable of transmitting the causative organism of pink eye (*Haemophilus aegyptius*, Koch-Weeks bacillus) from rabbit to rabbit under experimental conditions. Tashiro and Schwardt (*101*) noted *Hippelates* feeding on wounds inflicted by tabanids. Roberts (*82*) and Roberts et al. (*83*) showed that *Hippelates* could be associated with transmission of anaplasmosis. Other Chloropidae of importance are *H. flavipes* Loew (as *pallipes* Loew; see *87*, *88*) as a vector of yaws (*Treponema pertenue*) in the West Indies (*76*), and a *Hippelates* (species not given) as a vector of bovine mastitis (*Streptococcus agalactiae* Lehmann and Neumann) in Florida (*89*).

The genus *Hippelates* has also been implicated in transmission of the trypanosome responsible for pinta (Mal del Pinto), a disease common in the Americas south of the United States (*97*), and in acute nephritis in Trinidad (*6*, *7*). *Hippelates* has also been found carrying a variety of bacteria (*93*). Larvae of Chloropid flies (*Batrachomyia* spp.) are subcutaneous parasites of a number of species of frogs in Australia. Zumpt (*110*) listed 10 species of this Australian myiasis-producing genus.

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[1]Entomologist, Western Insects Affecting Man and Animals Laboratory, Science and Education Administration, Fresno, Calif. 93727.

[2]Italic numbers in parentheses refer to Literature Cited, p. 18.

Disease organism transmission by Chloropidae is thought to be mechanical (*79*) rather than biological; however, this does not affect the importance of these insects in those situations where they are the primary vectors. Though these insects do not have piercing-sucking mouthparts, the important *Hippelates* and *Siphunculina* species are strongly attracted to secretions from eyes, genitalia, or sores. Hence, the insects serve, as flying "cotton swabs" that move disease organisms, which are otherwise poorly capable or incapable of dispersion. Graham-Smith (*34*) described minute spines projecting above the pseudotracheal membrane of the mouthparts. He regarded these spines as capable of acting as cutting instruments that produce minute multiple incisions "...likely to assist pathogenic organisms carried by the insects in gaining a foothold."

Though adult eye gnats have long been known for their irritating habits, their breeding and larval developmental sites were not known until well into the 20th century. Much effort was expended toward the discovery of breeding sites in the United States, Egypt, India (Assam), Trinidad, and Jamaica. These gnats lay their eggs in earth, especially disturbed, friable soil, and the larvae utilize decomposing organic material for their nutrition.

Adult eye gnats are readily attracted to decomposing materials, of which various fermented chicken egg or liver preparations have been particularly successful. Many trap designs appear in published studies, some for survey purposes (for emergence as well as free-flying populations) and some intended for the reduction of eye gnat populations.

The discussion that follows will emphasize field techniques that have been described for New World *Hippelates*, but will also include the very few techniques mentioned in the comparatively sparse literature on Old World *Hippelates* and *Siphunculina*. It is a more complete account of the subject than is given in Rogoff (*85*) or in Kuitert (*53*).

EGG COLLECTION

Taylor and Olinger (*102*), in Florida, collected eggs of *Hippelates pusio* and *H. bishoppi* by use of a soil sampler, consisting of a copper pipe, 8 inches long and 4 inches in diameter, which was driven several inches into the ground. The top half inch of soil was removed with a spoon and placed in a labeled pint jar. "In the laboratory samples from each jar were screened into a pan, and mixed with water containing magnesium sulfate (3 to 1 by volume) to increase buoyancy of the eggs. The mixture was thoroughly stirred and allowed to settle for 1 to 2 minutes. The supernatant water was decanted through a sieve with 0.44-mm. openings. The eggs and debris that accumulated in the sieve were washed to remove the scum, and then transferred into another pan containing water and magnesium sulfate. The liquid phase was poured into pint jars, and the eggs were counted under a binocular microscope." Eggs were successfully collected from vegetable fields, citrus groves, and flowerbeds, but, in each situation, only from comparatively loose soil.

LARVA COLLECTION

Several techniques have been used to separate larvae from soil collections. Burgess (*13*), in the Coachella Valley of southern California, separated larvae

(probably *Hippelates collusor*) from soil by washing through a set of graduated screens. He found most specimens in the upper 4 cm of soil. Hummadi and Defoliart (*37*), in Wisconsin, processed soil collections in a Berlese funnel without success, but found larvae by washing soil through a series of sieves. They found most larvae of *Hippelates pallipes* and *H. bishoppi* from 5 to 7.5 cm from the surface.

Mulla (*64*), in California, studied the vertical distribution of larvae in artificially infested, 1-pint jars. Sections were removed from the soil columns in the jars and then washed with 100 milliliters of water. The supernatant was then vacuum-filtered through a black cloth in a Buchner funnel. This process left a few of the larvae and most of the pupae available for counting on the black cloth. The same portion of soil was then washed with 150 ml of 30-percent glycerine in water, and the supernatant was filtered as before. This second process floated out almost all the remaining larvae and pupae for counting on the black cloth. Mulla stated that: "Glycerine solution has advantages over salt solutions for floating out eye-gnat larvae. In the former, the delicate larvae can remain for some time without being damaged or killed, and live larvae are much easier to detect and count than those that are dead or moribund."

The various authors who have searched for breeding places of *Siphunculina* have not been helpful in describing their procedures for collection of larvae, though they expended considerable effort in the early part of the century (see Antonipulle (*1*), Ayyar (5), David (*14*), Hamilton (*35*), Roy (*86*), and Syddiq (*100*)). David (*14*) experimented with various oviposition and larval media and successfully colonized *S. funicola* on decomposing fish.

PUPA COLLECTION

The graduated sieve technique used by Burgess (*13*) and by Hummadi and DeFoliart (*37*), and the flotation method used by Mulla (*64*), for recovering larvae were also used for collecting pupae from small soil samples. Most pupae (*Hippelates collusor*) recovered by Burgess (*13*) or by Mulla (*64*) were in the upper 3 to 4 cm of soil, as were the pupae (*H. pallipes* and *H. bishoppi*) recovered by Hummadi and DeFoliart (*37*).

Mulla (*67*), in California, developed a technique for handling large volumes of material. He utilized a 25-gallon cylindrical tank provided with a water hose and valve to permit the flow of water into the tank and an outlet 1 inch below the inlet valve level. "The tank was filled with water, the soil samples were poured gradually into the water, and the soil-water mixture was stirred and then allowed to settle for a few minutes...pupae, puparia, and light debris remained at the top of the water.

"After the settling period a stream of water was allowed into the tank to flush the floating material through a 2 x 6-inch galvanized sheet-metal tube attached to the outlet. This material was caught on a series of 8-mesh, 16-mesh, and 35-mesh screens. The residues from the screens were washed into white enamel pans and the pupae or puperia were removed from the pans."

Large soil samples (100 liters/hr) were processed in a "soil-wash puparia extractor" by Bay (*8*) in California. The Bay extractor can be operated by inexperienced personnel and is claimed to be considerably less laborious and more efficient than multiple sieves and pans. Photographs and details of construction

and operation of the extractor are provided in Bay's article. By modifying screen sizes, the Bay extractor can be adapted to separate and concentrate other soil-inhabiting arthropods.

Most reports of larval *Hippelates* habitats concern loose, friable soil where a cover crop has been turned under. Bigham (*11*), working primarily with *H. pusio* and *H. bishoppi* (*26*), noted that in the southeastern United States gnats were abundant in regions of sandy or mucky soils but not in regions of dense, heavy or clayey soils. In terms of the organic matter of plant origin turned into the soil, Dow (*23*), in the southeastern United States, found that "...as more vegetation was turned under, conditions became less favorable for the production of *H. dissidens* and more favorable for *H. pusio*."

Siphunculina funicola habitats have included decomposing organic matter, particularly in the damp, soiled earth around improperly kept pail latrines and badly kept cattle sheds (*100*), in grass thatch of houses (*35*), and in soil heavily contaminated with fish offal (*1*).

ADULT COLLECTION

Many systems devised for the collection of small flying insects can be adapted for collecting Chloropidae. The most obvious of these, of course, is hand netting. For example, Dow and Hutson (*24*) netted several species, but mostly *Hippelates pusio*, from cows or hogs. Spielman (*98*) netted *H. pusio* from stabled horses. Mulla and March (*71*) netted *H. collusor* from around a human face; they noted that only female gnats were collected by this procedure. Legner (*56*) made net sweeps from vegetation as well as from the air immediately surrounding the heads of humans. He provided behavioral and aggressiveness data on some 17 chloropid species as well as on many other species of Diptera under 4 mm long at diverse sites in the Americas, the Mediterranean area, and east Africa.

Aspirators

Aspirators of various designs have been widely used for adult chloropid collection. Snow (*96*), in Alabama, collected *Hippelates pallipes* and *H. pusio* by aspirator from the giant star flower (*Stapelia gigantea* N. E. Br.). Dow and Hutson (*24*) aspirated *H. pusio* from man and other mammals, as did also Jay (*44*) and Thompson (*103*). Womeldorf and Mortenson (*108*) made collections from their persons and inside buildings and automobiles; their collections were mainly of *H. collusor*, but also included *H. pusio*, *H. robertsoni*, *H. dorsalis*, and *H. microcentrus*. Legner and Bay (*58*), collecting primarily *H. pusio* and *H. flavipes* on four West Indian islands, utilized a system of bait "...set inside an automobile with all windows closed except one. Both male and female gnats could be aspirated from closed windows as they flew into the automobile. Although females greatly predominated among the gnats attracted, a more balanced sex ratio could be obtained for culture by selectively collecting males from the windows. Largest catches were made in the early mornings or late afternoons when wind velocity was under 5 m.p.h., and in the complete absence of rain;...other species of Diptera that were attracted to the bait were easily excluded from the eye gnat collections by avoiding them during the aspiration process."

Roberts (*81*) utilized "...a portable 12-v. vacuum cleaner. A 24 x 24 mesh screen cylinder was inserted in the extension tube of the vacuum cleaner to retain the insects.... The vacuum cleaner was operated on the power supplied by the 12-v. battery of the truck.... After collection the screen cylinder was inserted into a killing jar and the contents emptied." A variety of Diptera, including four species of *Hippelates*, were taken within a steer-baited trap with this equipment.

Hippelates collections were made in the West Indies by Bassett (*7*) who utilized a batter-powered hand-held vacuum clothes brush, modified from the sort described by Husbands (*38*). Bassett's modification consisted of "...adding a nozzle; this was a piece of perspex tubing about 15 cm. long and 2.5 cm. in internal diameter. A long nylon net bag was fitted inside this nozzle, with the end of the bag folded back outside the tube and held in place by a rubber band. The machine readily drew flies into the bag, but some escaped by walking against the air flow to reach the open end. The end of the nozzle was therefore blackened so that the flies were attracted to the brighter light at the closed end of the bag. In this form the machine was used to make multiple catches.

"To catch *Hippelates* for bacteriological examination, smaller tubes were used. These were about 6 cm. by 1 cm., with a bung at one end and a disc of nylon net sealed across the other end. These tubes were mounted in a bung set in the nozzle of the machine. Flies were caught singly in these small tubes, kept in them as long as desired, and immobilized by introducing carbon dioxide through the nylon net before removal. The tubes were sterilized between uses."

The mechanical aspirator of Husbands (*38*), referred to by Bassett (*7*), had been previously further refined by Husbands and Holten (*39*), and though designed for mosquito collection, it could easily be adapted for collection of Chloropidae. Axtell (*3*) and Gerhardt and Axtell (*32*) also utilized a batter-powered vacuum aspirator for collection of *H. pusio* from human subjects.

Mulla et al. (*72*) assessed the density of *H. collusor* "...by collecting samples with a D-Vac..." as described by Dietrick et al. (*17*) "...from ground having grass and weed cover (where the gnats rest) at night. The eye gnats are inactive after dark and are not influenced by the presence of humans or other hosts. The samples were placed in 1/2-gal black painted ice-cream cartons provided with a funnel and collection vial at one end. Living insects ... moved readily into the collection vial, pretreated with a quick knockdown insecticide."

An aspirator for rapid handling of eye gnats in the laboratory was described by Schwartz (*90*). His apparatus represents a considerable improvement over the simple rubber-bulb aspirator for minute insects described by Kirkpatrick (*52*) and the somewhat more elaborate, electrically powered aspirator of Shanks and Gans (*91*).

Traps

Traps have been widely used to capture free-flying adult eye gnats for survey or control, for collection of disease vectors, or as emergence traps to monitor breeding sites.

Collection for survey or control.--Herms (*36*) stated that D. C. Parman, in California, had utilized various types of traps for free-flying *Hippelates* "... such as glass box traps and glass jar traps of various sizes baited with such material as hog liver and kidney of various ages, beef slime, blood, and sundry chemicals."

Parman (*77*, *78*) provided detailed plans for the construction of a box-type trap for eye gnat control. Parman's trap was 48 inches wide, 74 inches long, and 103 inches high. A bait vat and a wind-operated bait agitator were included in the design.

Burgess (*12*, *13*), in California, continued work with the trap described by Parman and devised many other, more easily constructed units. Burgess (*12*) pointed out that "Very efficient traps may be constructed from almost any available materials as long as the following features are included: (1) A darkened bait chamger or entrance chamber, (2) a good bait, and (3) a glass jar or other 'light chamber,' the entrance to which is at or near the top of the bait chamber."

Many of Burgess' experiments with various trap modifications were presented only as official reports, which are not generally available. Figures 1 to 8 are reproduced directly from Burgess (*12*). His description of the figures follows:

"Symbols applying to all figures:

a. Bait chamber.

b. Entrance to bait chamber.

c. Light chamber.

d. Standard for lean-to trap (optional).

e. Entrance to light chamber.

f. Awning over entrance to bait chamber in the lard can trap, formed by bending upwards the cut-out used to form entrance.

"Description of figures:

Figures 1, 2 and 3 are the front, side and rear views respectively of the lean-to trap. This is constructed as follows:

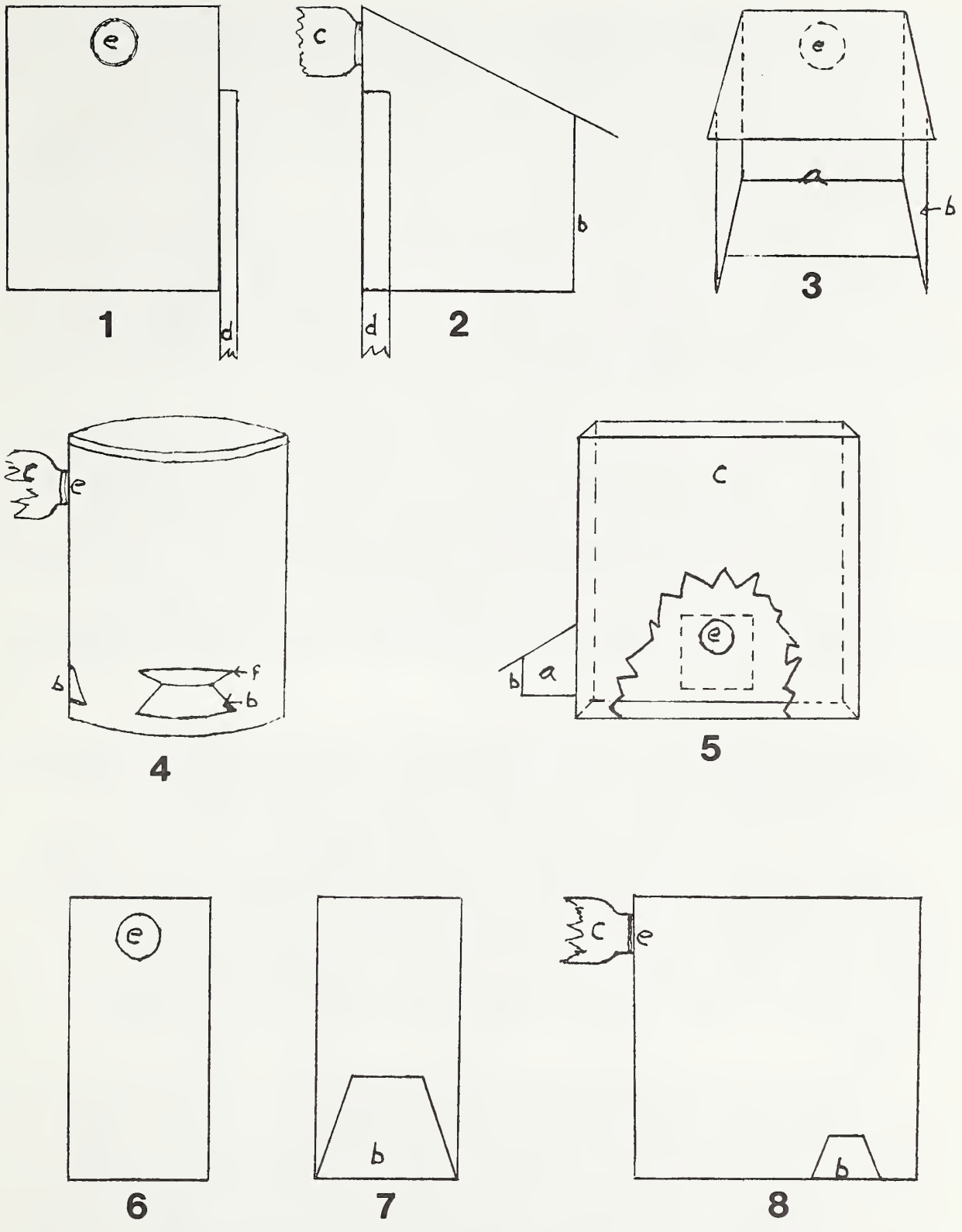
Front, 8" high by 6" wide, a hole bored at top center to fit half-gallon mason jar lid.

Sides, 8" high in front, 6" high in the rear, 6" wide at bottom.

Top, 8" long, 6" (plus twice the thickness of the material used) wide.

Bottom, 4" long, 6" (plus twice the thickness of the material used) wide.

Standard, a piece of 1" x 3" wood 4 feet long may be nailed to one side as a support if desired.



Figures 1-8.--Simple traps for catching eye gnats (from Burgess *12*).

"This provides a small trap into which a coffee can or other bait container may be inserted through the opening or entrance in the rear. No doors, baffles or other constructions are necessary.

"Figure 4 shows the construction of a trap from a lard can. Holes 4" wide at the bottom, tapering to 2" at the top, may be cut near the bottom of the can. The cut-out tin may be left intact along top of the hole and turned up to form an awning (f). Bait is placed directly in the bottom of the lard can.

"Figure 5 is a miscellaneous trap that may be constructed from an old box or barrel. Naturally, since the box is also the light chamber it must be covered with glass or very fine (60 mesh) screen. It must also be of a tight construction to prevent the escape of the gnats. The entrances in this case are also the bait chambers and are constructed exactly like the lean-to trap, except on a smaller scale, varying in size with the size of the box or barrel used.

"Figures 6, 7, and 8 show the front, side, and rear of the simple box trap. This trap consists of a wooden box (used as a bait chamber) 6" wide x 12" long x 12" deep. Unless this is used with a standard similar to that suggested for the lean-to trap, no bottom is required. A hole large enough to hold the lid of a half-gallon mason jar is bored in the top of the center of the front. The entrance holes are truncate inverted V-shaped holes cut in the rear and sides as illustrated. The side holes may be cut about 4 inches at the bottom and about 3 inches deep, leaving the top 2 inches wide. The rear hole may be 4 inches deep and 4 inches across the top. The size of these holes may vary without harm."

Bigham (*1*), in his surveys in Florida and Georgia, used traps based on the small units of Burgess (*12*). "Each was mounted on a stand covered above with a cloth canopy and had a half-gallon fruit jar for a light chamber."

Rees (*80*), in California, collected adult gnats by means of traps that "...consisted of one half quart fruit jars into which baits of various types were introduced. A fine mesh screen funnel with an apical opening just large enough to allow the passage of a gnat was inserted into the mouth of each jar. The whole was then capped with standard window screening, the screen and funnel being held in place by the open fruit jar lid. The arrangement permitted small flies, such as *Hippelates* spp., to enter, while at the same time preventing large flies from reaching the bait. After entering, the funnel prevented the gnats from escaping."

Tinkham (*104*, *105*), in California, utilized what he referred to as a "Tinkham trap" as well as a "modified Burgess trap." The Tinkham trap was a simplified device based on the principles enumerated by Burgess (*12*), except that it was elevated to "head height." It consisted of a darkened metal cone supported on a stand. The apex of the cone, which was attached below a glass container, was directed upward. A bait container was supported within the cone. This device served as the archetype of several more recent traps that were acknowledged as "modified Tinkham traps." Magy and Lee (*61*) and Hummadi and DeFoliart (*37*) utilized the Tinkham traps apparently in the original form. See also Womeldorf and Mortenson (*106*, *107*, *108*).

Womeldorf et al. (*109*) illustrated and described their modification of the Tinkham trap used in California. It "...possessed a truncated cone of sheet metal approximately 11 inches high, 12 inches in diameter at the base, and 2-1/2 inches in diameter at the apex... The upper end of the cone was fitted snugly into a tapered opening in a plywood frame and then into a 2-13/16 inch hole in a wooden block attached to the frame. Fitted tightly into the hole from above and held securely by its jar ring was the collecting chamber, an inverted one pint narrow-mouth Mason jar, provided internally with an inwardly-directed funnel of 30 mesh copper screen. The mouth of the jar was covered with 13 mesh nylon fabric to exclude large insects; both the nylon fabric and the screen funnel were held in place by the jar ring. The bait container, a one pint wide-mouth Mason jar, was suspended on a cross-wire inside the cone by means of wire hooks soldered to the jar ring. To prevent the gnats from drowning in the bait, the mouth of the jar was covered with fine mesh nylon screening cut from ladies' hose. Each trap was fastened to a tree, post or building at a height of four or five feet above the ground." Roberts et al. (*83*), used an undescribed modification of this trap.

The modification of the Tinkham trap used in California by Mulla (*68*) was illustrated by a photograph. It consisted of "...a sheet-metal cone and wooden flap fitted with a quart fruit-jar ring. The ring, fitting over the cone, is provided with a 40-mesh strainer-cloth cone supplied with a 16-mesh screen grid. The fruit jar is screwed tightly into the ring. Bait is placed in a polyethylene bag fitted into a 1-pint ice cream carton. The bait container is placed over a wooden support held by a hook attached to the main part of the trap inside the sheet metal cone." This same system was used by Mulla et al. (*72*).

Dow and Hutson (*24*), in southwest Georgia, utilized wooden traps based on "the simple box trap" of Burgess (*12*) as well as metal traps of their own design. They stated:

> "Four modifications of the Burgess box trap were developed in the Lower Rio Grande Valley of Texas in the course of studies made in 1947 by Mr. George B. Vogt and Mr. Judson U. McGuire. One improvement was to place a funnel of cellulose acetate in the mouth of the collecting jar to hinder the escape of the trapped gnats. The second change, made practical by the introduction of the funnel, was to mount the jar upside down on the top of the trap. The third was to place the bait dish on a shelf directly under the collecting jar. The fourth was to put cotton in the bait dish to prevent the drowning of gnats in the bait. At the Thomasville Field Station, one further change was made. The shelf for the bait dish was extended to the front of the trap and the large hole in the front half was covered with ordinary house screening (readily penetrable to gnats) in order to keep large flies out of the collections.
>
> "There were various objections to the modified Burgess box trap. Though the changes apparently increased the ability of the trap to retain gnats, they also limited the amount of bait odor which could escape to the outside. A second criticism was the difficulty of maintaining unpainted, wooden traps for use in an area as moist as southwest Georgia. A third objection was the probability that, with the box in a fixed position, the wind direction would strongly affect the amount of bait odor released. Finally, it was later realized that the distribution of the catch values was extremely skewed...."

The metal trap developed by Dow and Hutson (*24*) surmounted the objections to the box trap. The metal trap "...was made from an old-fashioned tin-plated dinner pail, 5 inches high and 7 1/2 inches in diameter. The entire bottom was cut out of the pail, and replaced by a horizontal partition of ordinary house screening soldered into place about 1 1/2 inches from the bottom. This served as a support for the petri dish, 20 mm deep, which was used for bait. Three legs, of 3/16-inch rod, were soldered on the outside. A conical collar of sheet metal, 2 3/4 to 3 1/4 inches in diameter, was soldered to the margin of a hole, 2 1/2 inches in diameter, cut in the cover of the dinner pail. This collar helped to hold in place the inverted one-pint glass fruit jar, used as the collecting chamber, which actually rested on the rim of the hole in the cover. All of these metal parts, except the screening, were painted with aluminum paint. A funnel was made of cellulose acetate with a hole at the apex about 3/16 inch in diameter. It was originally fitted to the mouth of the collecting jar, later cemented to a jar lid from which the center had been removed, and, finally, rigidly bound between two rings of metal, also cut from jar lids. This metal-rimmed cone was held on the jar by means of the threaded ring manufactured to hold the lid, and needed only to be plugged with cotton to hold the catch of gnats for removal to the laboratory."

Jay (*44*), in Florida, also used simple survey traps that "...consisted of 1/2-gallon Mason jars placed on the ground in a horizontal position. Each contained a gauze-covered 50-ml beaker..." containing the bait. "A piece of 14-mesh screen was cut to fit inside the ring of the jar to allow only the smaller insects attracted to the bait to enter."

Spielman (*99*), in Cuba, utilized easily constructed survey traps "...fashioned from unpainted number-10 cans, each fastened at one side to a stake at a height of 18 inches. Three equally spaced, 1"-square openings were cut in the sides near the bottom of each can, and a similar hole was made close to the top edge opposite the stake. A dieldrin-treated pint jar, with a 16-mesh screen disk in its mouth, was attached to the latter opening, which always faced south. The metal bottom of the can was removed and replaced by a wooden door that served to hold the bait."

Thompson (*103*), in New Jersey, utilized simple conical traps for sampling Diptera. These traps were made from hemispherical sections of plastic "...63 inches long... Clothes pins about the base of the cone raised each trap about 2 in from the ground and allowed exit of CO_2 and entry of the insects... A 1-in hole was cut from the apex of the cone to allow mosquitoes to enter a Mason jar resting on top of it. A small Mylar cone, also with an apical aperture, was taped within the metal rim of the Mason jar to permit entry...but to exclude... exit."

Legner (*56*, *57*), for surveys in various parts of the world, developed an easily transportable bait trap which consisted of two vials approximately 3 cm in diameter. The darkened lower vial, about 8.5 cm tall, served as a bait receptacle and had screen (plastic, 6.1 mesh/cm) openings cut into the sides near the top. The clear upper vial, about 6.5 cm tall was inverted over the bait receptacle. The original caps of the two vials were perforated and cemented together and included an inverted funnel that projected into the collection vial through a slit in a piece of organdy. The latter permitted disassembly without loss of the insects collected. The entire apparatus was commonly suspended from a tree limb with a barrier of grease to prevent ant depredation.

Snoddy (*93*) studied the comparative "...efficiency of colored 3M Sectar I™ (Manufactured by 3M Corp., Minneapolis, Minn.) disposable insect traps...." and "...the standard Coastal Plain Experiment Station (CPES) traps... The CPES traps are miniature 'light' traps, without light, redesigned...." from the CDC miniature mosquito light trap. The CPES traps outperformed the best of the colored 3M traps (red) by a factor of about 2.5.

Collection for pathogen isolation.--Defoliart and Morris (*15*), in Wisconsin, devised a trap for the collection and field storage of hematophagous Diptera. This trap collected a wide variety of species including *Hippelates bishoppi* and *H. plebejus* (*16*). The captured specimens "...slid, while still alive, into a dry ice chamber, and the frozen collections were removed at 2- to 3-day intervals. Material thus collected furnished information on seasonal occurrence of the Diptera and was suitable for attempted arbovirus isolations."

Detailed plans for the construction of the trap are provided in the article and illustration (fig. 9) by DeFoliart and Morris (*15*) and are summarized as follows: "The trap consisted of three main parts...an insulated container (A) for storage of dry ice, the CO_2 from which was released through a small perforation on each side; a large cone (B) placed over the dry ice container, the cone having 4 large openings on its basal periphery for entry of attracted Diptera, and a small opening at its apex for their exit into the catch chamber; and a catch chamber (C) shaped as a downward-directed funnel with sealed cover from which trapped Diptera slid down a tube to cold storage within the dry ice container."

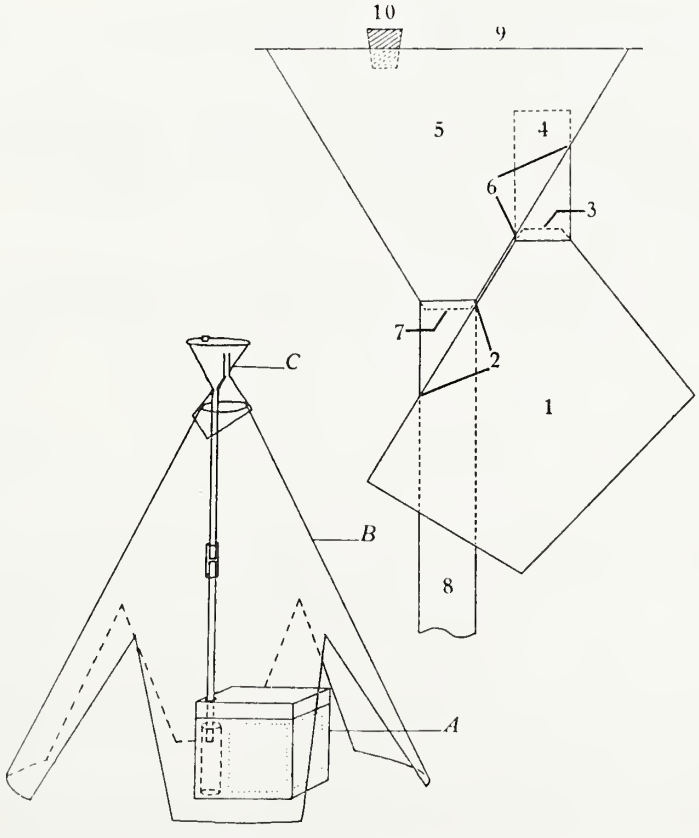


Figure 9.--Dry ice-baited trap (from DeFoliart and Morris *15*).

In his studies at the laboratory of the Jamaica Yaws Commission, Kumm (*54*) caught *H. flavipes* "...in large bore glass tubes after they had fled to repletion on various types of yaws lesions...and they were transported in these tubes to Kingston for subsequent dissection."

Traps for Field Tests of Candidate Attractants

Snoddy (*92*), in Georgia, devised a simple, disposable trap for testing series of candidate attractants in the field. This trap consisted of four 6-oz paper cups with the bottoms removed "...and a 3" by 5" index card stapled inside. A 3" by 3" area of the card was coated with Stickem." These traps were hung from suspended, 24-inch wood lath crossarms.

Dow (*21*) described and pictured a "gnat-trap rotary" designed for simultaneous trapping in field tests of potential attractants. This apparatus provided slow, horizontal rotation of the test items, carousel-fashion, around a central point. A turntable, 4 ft in diameter, supported the metal gnat traps and also served as the pulley for a belt bringing power from a motor about 10 ft away.

Mulla et al. (*73*) devised a more elaborate olfactometer, for field use, with one component based on a modification of the Tinkham trap, and the other similar to the "gnat-trap rotary" of Dow (*21*). Mulla designated his device as the "C.E.S. Eye Gnat Olfactometer," the "C.E.S." in reference to the Citrus Experiment Station of the University of California. Portions of his description follow:

> "This olfactometer consists of four main parts, as described below.
>
> "1) *C.E.S. Eye-Gnat Trap.*--This trap is made of a 100-mm. Pyrex fluted clear funnel with a 7-dram display glass vial as a collection chamber... The stem of the funnel is cut off about 10 mm. from the neck...
>
> "The collection vial is fitted with a polyethylene closure from which the hard, inelastic supporting disk is removed. A small hole equal to the outer diameter of the stem of the funnel is drilled in the center of the closure. The closure can be fitted easily into the vial, and the collection vial can then be inverted and slipped over the stem of the funnel. The polyethylene closure fits tightly over the funnel a few millimeters below the neck line.
>
> "2) *Bait Dish.*--The bait dish is placed under the trap over the screened portion of the olfactometer...
>
> "3) *Turntable.*--The turntable...is made of high-grade exterior plywood 1/4-inch thick. It is circular in shape with a diameter of 4 feet. A 7-inch turntable disk is fastened in the center of the turntable; a hole in the center of the disk with a set screw accomodates the 1/2-inch vertical shaft from the reductor.
>
> "Twenty circular holes, 2 inches apart, are cut into the turntable periphery. The diameter of the hole is equal

to the outer diameter of the funnel so that the latter can be inverted over the hole and the screen support, as described below...

"Pieces of household window screen (16-mesh) or hardware cloth (8-mesh) are stapled over the holes on the underside. These screen disks act as supports for the funnel trap and also keep insects that are much larger in size than the eye gnats from entering the trap...

"4) *Power Unit, Speed Controller, and Reductor*.--The housing below the turntable...contains an electric motor..., a...variable speed torque converter...and a...gear reductor.... An 18-inch aluminum rod 1/2 inch in diameter...coupled with the output shaft of the gear reductor...acts as a support and shaft for the turntable." This same olfactometer was briefly redescribed by Hwang and Mulla (*41*).

Beavers et al. (*10*), in Florida, tested a series of esters and acids as candidate attractants in sticky traps "...prepared by coating the inner surface of 1-qt. ice cream cartons with Stikem, cutting away the upper 1/2 of each end of the carton to allow entrance into the trap, and suspending a dental roll (1 in. long, 3/8 in. diam) containing 1 ml of the test compound inside the carton ca. 2 in. below the upper surface. The traps were suspended horizontally ca. 6 ft. above ground and spaced 75-100 ft. apart for [a] 3-4 day/test."

Rogoff et al. (*84*), in California, tested a series of candidate attractants in traps of a modified Tinkham design, borrowed from the Coachella Valley Mosquito Abatement District, where they had been in use for many years, having been designed by A. L. Cavanaugh (*62*). These sturdy traps were constructed of a heavy gage sheet metal. A central cylinder, 10 cm in diameter and 23 cm long, was surrounded by an apronlike sheet, formed as a distorted frustum. The latter was painted black on its inner surface and served as the entrance chamber. Bait was placed in the lower part of the cylinder. Insects entered through holes in the cylinder within the entrance chamber. A pint Mason jar fitted with a fine-screen cone and a coarse-screen barrier served as the light chamber; it was inverted and placed on a shelf in the upper part of the cylinder. In use, the traps were hung on metal poles driven into the ground.

Baits

Collection of adult gnats in other than emergence traps has generally utilized some form of attractant in conjunction with the mechanical device. These have generally consisted of empirical mixtures of various types of decomposing organic matter, though some attempts have been made to discover and use synthetic materials.

(1) *Naturally derived baits*.--Herms (*36*) noted the use of "...hog liver and kidney of various ages, beef slime, blood and sundry chemicals." Parman (*78*) recommended a mixture of "...1 pound of beef or hog liver and 2 ounces of urea to 4 gal of water." Parman was working with large traps and recommended the use of 1 pint of bait to each cubic foot of the trap. Kumm (*55*) used the liver-urea mixture in the proportions stated by Parman (*78*). Burgess (*12*) asserted: "Many

substances, both chemical and organic, have been tried as baits. Of all these, liver bait is most efficient."

Bigham (*11*) used a bait that "...consisted of beef liver cut into small pieces, 1 teaspoonful of urea, 1 teaspoonful of table salt, and enough water to make 1 pint. It was kept in a sealed fruit jar until ready for use." Burgess (*13*) stated that the bait, finally adopted as standard in the California Coachella Valley activities, "...was composed of hog liver, urea, table salt, adobe soil and water. The dilution could be varied to fit the requirements of different traps and location." Jones and Magy (*46*) also used the liver-urea-water type bait.

Tinkham (*104*) used an egg bait (fresh or rotten egg in water) for *Hippelates* trapping in California's Coachella Valley. Rees (*80*), in California's San Joaquin Valley, also utilized egg baits (albumen, albumen and yolk, or yolk) as well as "...fish meal, blood meal and finely chopped and putrifying liver."

Dow and Hutson (*24*), in southwest Georgia, for surveys of *Hippelates pusio*, used a bait which "...was always made of pork liver. The basic formula was: 1 lb. of ground liver, 1 lb. of water, 1 oz. crystalline urea. Put in glass fruit jars and allowed to putrify at room temperature, the mixture appeared to reach a stable condition in about 2 weeks. By preparing many small batches, thoroughly mixing all the successful lots, and deepfreezing this master mix in suitable containers, it was possible to have a bait that was uniform for the duration of a long study...each trap was baited with...about 25 ml...poured on a layer of cotton in a petri dish." This same bait formula was used by Dow (*22*).

Dow (*21*) found putrified pork liver bait significantly superior to egg bait (raw egg in water, after the manner of Tinkham) whether or not the egg bait was allowed to putrify. He also found that the liver bait allowed to putrify under aerobic conditions was more attractive than bait matured anaerobically.

Mulla (*63*), with his C.E.S. Olfactometer in southern California, reported that "Among the proteinaceous substances, solutions or preparations of whole egg powder, egg yolk, egg albumin, lactalbumin peptone, and partially hydrolyzed yeast were highly attractive to..."*H. collusor*"...corn protein, fish meal, and gelatine were slightly attractive."

Mulla et al. (*74*) stated: "Aerobic fermentation at 88 ± 2° F. was found to liberate the attractant principle, at various rates and time-intervals of aging, from various materials exposed and tested in the eye-gnat olfactometer. Fresh preparations of these materials manifested little or no activity. Attractancy of Staley's Corn Protein Bait No. 7 was highest at the end of a 19-day test period; of Staley's Corn Protein Bait No. 2, on the 13th day; of lactalbumin peptone, lactalbumin hydrolysate, and Edamin, on the second day; of autolyzed yeast, brewers yeast, and partially hydrolyzed yeast, on the third, fourth, and seventh day, respectively. Egg albumen was highly attractant around the fourth day of aging, whole egg and egg yolk on the tenth and the sixth day, respectively, though the peak attractance of the last two to *Milichiella* sp. was indicated around the second day. Homogenates of fresh *Hippelates collusor* and *Musca domestica* were also attractants for eye gnats, but dried *H. collusor* homogenized into water showed no appreciable attractancy. With lactalbumin peptone and whole egg, there was a steady increase in the capture of eye gnats as the concentration of the materials was increased; but with autolyzed yeast this was true only for concentrations up to the 0.5% level."

Jay (*44*) used fish-baited traps in Florida as did Axtell (*2*) in North Carolina. Spielman (*99*), in Cuba, used "...a large, freshly caught shrimp which was replaced every morning." Womeldorf and Mortenson (*106*, *107*), in the San Joaquin Valley and in the Mohave Desert of California, used a stock bait solution "...prepared by breaking eight eggs into a container and allowing them to age several days. Water was then added to bring the total volume to 2 quarts. Each trap was baited every other week with 1 pint of the stock solution. On alternate weeks water was added to keep the volume constant."

Mulla (*68*) and Mulla et al. (*72*), in southern California, prepared bait "...by blending whole egg powder with water to give 2% suspension, or by blending four chicken eggs with a quart of water. The preparation was aged septically for 4 to 5 days before it was exposed in the traps. Bait was changed twice monthly during the warm months and once a month during the cooler months. Between changes, water was added to the bait container at each collecting time to replenish water loss due to evaporation."

In the West Indies, Legner and Bay (*58*, *59*) collected eye gnats "...by attracting them to a mixture of rotting fish and eggs in water."

Legner (*56*, *57*), for surveys in the Caribbean and in Central and South America, loaded 10 by 25 mm gelatin capsules with powdered egg and anchored two such capsules in the bottom of each of his traps. The bait was activated by the addition of 200 ml water and appeared to be most effective "...from the third through the sixth days after activation when temperatures ranged from 22 - 32° C."

Snoddy (*93*), in Georgia, used an eye gnat bait composed of "...a 10% pasteurized egg solution degraded with 1% pineapple enzyme, bromelain, at ca. 35° C. for 72 hr. Enzyme degraded egg was used in preference to the egg bait degraded by chance flora at room temperature because it produced more uniform trapping results..." Snoddy (*94*) evaluated five commercially available enzymes to determine their effectiveness on whole egg and egg albumen in affecting their attractiveness to *Hippelates pusio*.

Attempts to isolate and identify the attractant compounds in various fermented aqueous suspensions of whole egg powder were made by Hwang and Mulla (*41*, *42*). Mulla et al. (*75*) and Mulla and Axelrod (*70*) developed dry attractive baits "...obtained by drying of 10-35% (wt/wt) suspensions of whole egg solids in water, fermented for a time at 30-33° C...volatile attractants remain in the residue on freeze-drying and spray-drying." Hwang, et al. (*43*) isolated and identified many of the attractants and co-attractants of the fermented aqueous suspension of chicken whole egg powder.

(2) *Synthetic (identified) baits*.--Many investigators have attempted to develop synthetic attractants. Mulla (*63*) studied amino acid mixtures and, as previously noted, attempted to isolate the components of fermented egg mixtures as pure compounds (*41*, *42*, *43*). Snoddy (*92*) studied 60 carbonyl compounds and found that 2,3-pentanedione was attractive to members of the genus *Hippelates*. However, although *H. bishoppi*, *H. pusio*, and *H. pallipes* were taken, more than 95 percent of the specimens taken were *H. dissidens*, which is not considered a pest species. Fluno et al. (*29*) noted that as far back as 1963, several genera of Chloropidae (but no *Hippelates*) were taken with several yellowjacket lures, 2,4-hexadienyl butyrate (2,4-HDB), 2,4-hexadienyl propionate, and heptyl butyrate (HB). Beavers et al. (*10*), in a Florida study involving many similar compounds, successfully captured *H. pusio* with 2,4-HDB, but not with HB nor with 2,4-hexadienyl hexanoate, though these latter materials attracted other chloropid genera

(*Olcella* spp. and *Conioscinella* sp.). Rogoff et al. (*84*), in California, captured a small number of *H. collusor* with HB, but not with 2,4-HDB, though other chloropids, *Conioscinella flavescens* (Tucker) and *Siphonella neglecta* Becker, were readily taken with 2,4-HDB, HB, and many similar materials.

Defoliart and Morris (*15*) and DeFoliart et al. (*16*), in Wisconsin, captured *H. bishoppi* Sabrosky and *H. plebejus* Loew with carbon dioxide as the lure. Thompson (*103*), in New Jersey, also used carbon dioxide to capture *H. plebejus* and *H. nobilis* Loew. Dorner and Mulla (*18*, *19*), in California, found slight attractancy in a fresh homogenate of eye gnats, an increased effectiveness in a putrified homogenate, and slightly more attractancy in homogenates of female eye gnats than in homogenates of males. (See also *74*.)

Emergence Traps

Bigham (*11*), in Florida and Georgia, used "field recovery cages...to locate the breeding places of the gnats. Each cage covered an area of 1 square yard. The sides of the cage consisted of four boards, 1 inch by 12 inches, nailed together at the corners. The top was covered with 8-ounce duck. A hole was cut in one side near the upper edge and a pint fruit jar was screwed into this to form a light chamber. The cages were placed over suspected breeding places and were emptied once a week or oftener by spreading a black oil cloth over the canvas top and beating lightly on the cloth so as to drive the insects out into the fruit jar."

Tinkham (*104*, *105*), in California, used similar emergence traps that were 1 $yd.^2$ Rees (*80*), in California, used emergence traps similar to, but smaller than, those used by Bigham (*11*). Dow and Willis (*25*), in Georgia, utilized emergence traps modified from those used by Bigham (*11*). The inside dimensions of their rectangular traps "...were 2 x 3 ft. The sides of the trap, 12 inches high, were of tongue and groove boards, nailed to external corner posts 2 x 2 inches in cross section. The top of the trap was a sheet of white canvas. Nailed to diagonally opposite corners on top of the trap were two blocks of wood bored to receive the top of the inverted 1-pint glass fruit jars which served as collecting chambers. In the mouth of each jar was placed an inwardly directed funnel made of cellulose acetate. This acted as a trapping device and also held dead gnats in the jar. The funnel had an opening 3/16-inch in diameter at the apex, and its base was glued to the rim of a jar lid from the center of which a disk 2 1/8 inches in diameter had been removed. The funnel was held on the jar by the threaded ring (screw band) which is made by the manufacturer to hold the lid. Under the wide range of weather conditions to which the emergence traps were exposed, the cone would frequently loosen from its supporting ring. The remedy found for this difficulty was to bind the base of the cone rigidly and snugly between two rings of sheet metal cut from jar lids."

Mulla (*65*, *67*) utilized emergence cages similar to those of Bigham (*11*). Pine frames (1 yd^2) were covered with black cloth and canvas. A fruit jar fitted with a 40-mesh screen cone was attached 4 to 5 inches from the lower edge of each frame to serve as the collection chamber. Gaydon and Adkins (*30*), in South Carolina, also used traps of this type. They emphasized the importance of "...an inner black sheet to darken the interior and an outer white sheet to reflect

light and reduce heat buildup inside the trap." The Mulla (*65*) design was also used by Legner et al. (*56*) in California.

Jay (*45*) used emergence traps "...of two sizes, one enclosing 9 square feet and the other 4 square feet. They were made of Masonite, with a 4-inch galvanized metal plate attached around the bottom to facilitate easy insertion into the soil. They had flat roofs and a circular opening was cut in one side of each trap to permit the attachment of a fruit-jar lid. Two days after the traps were placed in the soil, 1-pint fruit jars were screwed into the lids on the traps. These jars served as the only entry for light into the interior of the traps. Newly emerged gnats, being positively phototactic, were attracted to these jars."

POPULATION DYNAMICS

Dispersal

Mulla and March (*71*) tagged *Hippelates collusor*, caught in the wild, with radioactive phosphorus (P^{32}) and released them in the Palm Desert and Indio areas of southern California. "The labelled gnats dispersed over an area of 4 + square miles within a period of active flight of 5 - 6 hours. (Gnats are inactive at night and maximum flight activity is noticed early in the morning and late in the afternoon.) After 13 days they had dispersed over about 10 square miles, an expanse representing approximately the total favorable habitat in this area."

On the basis of the ratio of tagged to untagged gnats in the recapture traps, it was possible to arrive at a rough estimate of the number of gnats in the area surveyed. The figures presented were in terms of 15 to 30 million or roughly 3,500 to 5,000 female gnats per acre. This computation did not take into account variations in preferred habitat. "Dispersal occurred both up-wind and down-wind, but the greatest distance travelled..." was somewhat over 4 miles with the wind.

A similar study was conducted by dow (*22*) in southwest Georgia. Dow found that P^{32}-tagged *H. pusio* could move upwind even on a windy day. Dow noted rapid dispersal of the gnats, and releases 1/2 and 1 mile "...from a rural population center...resulted in almost complete penetration of the small town on the day of the release. In one test, traps more than a mile from the release box caught 15 gnats in less than 3 1/2 hours after it was opened."

Responses to the Environment

Dorner and Mulla (*20*), in California, and Gerhardt and Axtell (*32*), in North Carolina, provided data on the effects of light, wind, moisture, and temperature on eye gnat behavior and capture.

Spielman (*99*) found population increases of *Hippelates pusio* and *H. impressus* Becker "...2 to 3 weeks after periodic rains in a non-agricultural, semiarid area in Cuba." In southern California, Mulla (*69*) demonstrated that *H. collusor* oviposition activity increased "...soon after the disturbance or disking of its

natural habitats. Most of the eggs were deposited within a few hours after the disking, and within 24 hours a great portion of the eggs were laid..." with no appreciable increase thereafter. "Peak emergence was within a period of 2 to 4 weeks after the disking."

Important laboratory data on the effects of population density and age on fecundity, fertility, and oviposition of *H. pusio*, *H. bishoppi*, and *H. pallipes* were developed by Karandinos and Axtell (*49*, *50*, *51*). Seasonal populations of *Hippelates*, in North Carolina, were provided by Axtell and Edwards (*4*), and correlation of flight with temperature, light, moisture, and wind velocity were given by Gerhardt and Axtell (*33*).

REARING AND HANDLING

No attempt will be made here to elaborate on the rearing or special handling of chloropids other than what has already been presented; however, some literature citations are in order. For the rearing of *Hippelates collusor*, *H. robertsoni* and *H. dorsalis*, see Mulla (*66*); for *H. pusio*, see Mulla (*66*), Jay (*45*), and Karandinos and Axtell (*47*, *48*); for *H. pallipes*, see Hummadi and DeFoliart (*37*) and Karandinos and Axtell (*47*, *48*); for *H. bishoppi*, see Jay (*45*) and Karandinos and Axtell (*47*, *48*). For *Siphunculina funicola* rearing, see David (*14*) and Hutson (*40*).

Mass rearing of *H. collusor*, *H. pusio*, and *H. robertsoni* is described by Mulla (*66*); of *H. collusor*, by Bay and Legner (*9*) and Eskafi (*27*); and of *H. pusio*, *H. bishoppi*, and *H. pallipes*, by Karandinos and Axtell (*47*).

Holding *H. collusor*, once captured, is discussed by Georghiou and Mulla (*31*) and by Flanders and Bay (*28*); *H. pusio*, by Dow (*21*) and Spielman (*99*); *H. bishoppi* and *H. pallipes*, by Hummadi and DeFoliart (*37*); *H. pallipes*, by Kumm (*54*); *H. robertsoni*, by Georghiou and Mulla (*31*); and *H. impressus*, by Spielman (*99*).

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